

HISTOCHEMICAL STUDIES ON THE DEVELOPING ABOMASUM OF GOAT IN PRENATAL PERIOD

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ABSTRACT

The present study was conducted on developing abomasum of prenatal goat from 32 to 150 days of foetal life. The foeti were divided into three groups (12 embryos/ foeti in each group) viz. Group I (age \leq 50 days), Group II (age 51-100 days) and Group III (age 101 - 150 days). The samples were stained for PAS, AMPS, Bound lipids, Alkaline and Acid phosphatase and DNA. Surface epithelial cells of pyloric region showed intense PAS activity than fundic part. AMPS and lipid activities were higher in pyloric region. Intense Feulgen reaction was noticed in the nuclei of nerve cells and smooth muscle cells.

Keywords: Abomasum, Fundic, Histochemistry, Pyloric, Prenatal goat

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Goats have an ability to convert fibrous foods into products of great nutritive value. The enzymatic hydrolysis of the food occurs in abomasum with the help of parietal and chief cells. The mucous secreted by surface epithelial cells and mucous neck cells forms an alkaline sheet over the epithelial surface which provides protection from gastric juice. The functions of these cells are influenced by histochemical properties of the epithelium and glandular cells. A great deal of research has been carried out in the histochemical reactions of abomasum during postnatal development but literature of prenatal study is lacking. The results of this study fulfill the lacuna of scientific literature of histochemical reaction of abomasal cells during intrauterine life.

MATERIALS AND METHODS

The present study was conducted on the developing abomasum of 36 healthy and normal embryos/foeti of either sex of non-descript goat (*Capra hircus*) collected from the local abattoir, cases of dystocia and abortion from clinics and farms. An approval was obtained from animal ethic committee of DUVASU, Mathura (U.P.) prior to the commencement of the study. The embryos/foeti ranged from 32 days to near full term. The age of embryos/foeti was ascertained by using formula derived by Singh *et al.* (1979) for goat foetus, $W^{1/3} = 0.096(t-30)$, where, W = body weight of foetus in gram and t = age of foetus in days. Embryos/foeti were assigned into three groups viz. group I (0-50 days of gestation), group II (51-100 days of gestation) and group III (101-150 days of gestation) containing 12 embryo/foeti in each group. Small pieces of tissues from fundic and pyloric part of abomasum were

collected in group II and III, while in group I whole of the stomach was collected. The tissues were fixed in 10% neutral buffered formalin (NBF) and cold acetone. Fixed tissues were processed by routine paraffin embedding technique and 6 mm thick sections were taken. The sections were stained for demonstration of Polysaccharides (PAS) (Periodic Acid Schiff's, Luna, 1968); Acid mucopolysaccharides (AMPS) [Muller's Colloidal (hydrous) ferric oxide, Luna, 1968]; DNA (Feulgen's reaction, Bancroft and Stevens, 1979), Lipids (Sudan Black B method, Pearse, 1968) and Alkaline phosphatase and Acid phosphatase enzymes (Gomori's method, Pearse, 1968).

RESULTS AND DISCUSSION

Polysaccharides (PAS): Surface epithelium of fundic part of abomasum showed intense PAS positive reaction in group I and this activity gradually decreased in group II and III (Fig. 1 & 2). Singh *et al.* (2012) did not find neutral mucopolysaccharides activity in fundic epithelium but noticed PAS positive neutral mucopolysaccharides in pyloric epithelium at 5.5-7.5 cm CRL stage in buffalo foetal abomasum. However, in present investigation reverse trend for PAS activity was observed in pyloric region. Supranuclear zone of surface epithelial cells had PAS positive granules in pyloric region (Fig. 5). Masot *et al.* (2007) in red deer reported neutral mucopolysaccharides in the epithelium of abomasum from 97 days of gestation. Cells of the gastric pit of fundic part exhibited weak to moderate PAS reaction in group II and III (Fig. 3) while, in the pyloric region moderately PAS positive granules were encountered (Fig. 4). This observation was in partial agreement with the findings of Singh *et al.* (2012). They

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observed PAS positive neutral mucopolysaccharides at the base of gastric pit of pyloric stomach between 5.5-7.5 cm CRL in buffalo foeti. In group III, cells of the body of the gland showed moderate PAS activity in fundic region while in pyloric part, the supranuclear zone of the cell showed intense PAS positive granules in group III. Cells of secretory end pieces of fundic as well as pyloric abomasum showed moderate to intense reaction on their luminal border in group II and III (Fig. 2, 4 & 5). Present observations regarding PAS activity in fundic epithelium and its gland were partially similar to the findings of Singh *et al.* (2012) in buffalo foeti. Ramkrishna and Tiwari (1979) reported maximum glycogen in pyloric part during early stage of development and in fundic region in late gestational period in prenatal goat. The activity found in mucosa of pyloric stomach and its glands were identical with findings of Singh *et al.* (2012) in prenatal buffalo. Whereas, reverse observations were made by Ramkrishna and Tiwari (1979) in goat. However, presence of granules found in pyloric part of epithelium and glandular cells could not be compared for the want of similar observations

in literature. Present observations were in full agreement with the observation of Pangtey *et al.* (2014) in human foeti. These authors observed PAS positive activity in fundus and body. The basement membrane showed intense PAS positive reaction in fundic as well as pyloric part of abomasum. In the fundic region, connective tissue elements of lamina propria and smooth muscle showed weak to moderate reaction in group II and activity decreased in group III whereas, in the pyloric region weak reaction was observed in all the groups (Table 1 & 2). Singh *et al.* (2012) observed mild PAS reaction in submucosa of buffalo foetal abomasum. Smooth muscle cells of lamina muscularis and tunica muscularis of fundic region showed moderate activity in group II which decreased gradually in group III, while in pyloric part activity was higher in group I and gradually decreased in rest of the groups. Singh *et al.* (2012) observed high PAS content in lamina muscularis and tunica muscularis at 211 days of gestation. Blood vessels showed moderate and intense reaction in fundic and pyloric part, respectively. Cells of neuronal elements showed weak to negative

Table 1

Histochemical reactions in fundic abomasum of prenatal goat at various stages of gestation

Group	Characters	Cytoplasmic Characters					Nuclear Character
		PAS	AMPS	Lipid	ALK	ACP	Feulgen reaction
I (0-50 days of gestation)	Surface epithelial cell	+++,+++*	+	++*	-	-	+ to ++
	Propria-submucosa	+,+++#	-	-	-	-	+++
	Tunica muscularis	+++	-	++	-	-	++ to +++
	Blood vessels	+++	-	++	-	-	+++
	Nerve cells	++ to +++	-	++	-	-	++ to +++
	Serosa	+++	-	-	-	-	-
	(mesothelium)						
II (51-100 days of gestation)	Surface epithelial cell	++,++*	++	++*	-	-	+ to ++
	Glandular epithelium	++ to +++	++	++*	-	-	++ to +++
	Propria-submucosa	+,+++#	-	-	-	-	+++
	Tunica muscularis	++	-	+++	-	-	++ to +++
	Blood vessels	+++	-	+++	-	-	+++
	Nerve cells	++ to +++	-	+= to +++	-	-	++ to +++
	Serosa	+++	-	-	-	-	-
(mesothelium)							
III (101 days of gestation to till term)	Surface epithelial cell	++,++*	++ to +++	++*	-	-	+ to ++
	Glandular epithelium	++ to +++	++	++*	-	-	++ to +++
	Propria-submucosa	+,+++#	-	-	-	-	+++
	Tunica muscularis	++	-	+++	-	-	++ to +++
	Blood vessels	+++	-	+++	-	-	+++
	Nerve cells	++ to +++	-	++ to +++	-	-	++ to +++
	Serosa	+++	-	-	-	-	-
(mesothelium)							

- Negative, + Mild, ++ Moderate, +++ Intense, * only in cell boundaries, # only in Basement membrane

Table 2
Histochemical reactions in pyloric abomasum of prenatal goat at various stages of gestation

Group	Characters	Cytoplasmic Characters					Nuclear Character
		PAS	AMPS	Lipid	ALK	ACP	Feulgen reaction
I (0-50 days of gestation)	Surface epithelial cell	+++ ,+++*	-	++*	-	-	+ to ++
	Propria –submucosa	+, +++#	-	-	-	-	+++
	Tunica muscularis	+++	-	++	-	-	++ to +++
	Blood vessels	+++	-	++	-	-	+++
	Nerve cells	++ to +++	-	++	-	-	++ to +++
	Serosa (mesothelium)	+++	-	-	-	-	-
II (51-100 days of gestation)	Surface epithelial cell	++, ++*	-	++*	-	-	+ to ++
	Glandular epithelium	++	+ to ++	++	-	-	+ to ++
	Propria –submucosa	+ to ++, ++#	-	-	-	-	++
	Tunica muscularis	++	-	++ to +++	-	-	++
	Blood vessels	++	-	+++	-	-	++
	Nerve cells	+	-	++	-	-	++ to +++
Serosa	-	-	-	-	-	+	
III (101 days of gestation to till term)	Surface epithelial cell	+++ ,+++*	+++ ,+++*	+++*	-	-	+ to ++
	Glandular epithelium	+++	++ to +++	+++	-	-	+ to ++
	Propria –submucosa	- To +	-	-	-	-	++
	Tunica muscularis	-	-	+++	-	-	++
	Blood vessels	++	-	+++	++	-	++
	Nerve cells	-	-	++	-	-	++ to +++
Serosa	-	-	-	-	-	+	

- Negative, + Mild, ++ Moderate, +++ Intense, * only in cell boundaries, # only in Basement membrane

reaction in fundic part in all groups, while moderate to intense reaction in group I and II and weak to moderate in group III in pyloric part. Mesothelium showed intense PAS reaction in serosa of both the regions in all groups.

Acidic mucopolysaccharides (AMPS): The surface epithelial cells of fundic as well as pyloric region showed weak to moderate reaction of AMPS which gradually became intense at full term (Fig. 6 & 7). Presence of AMPS may provide protection to the epithelium. Only supranuclear zone of chief cells exhibited moderate AMPS reaction while other cells of fundic gland did not show the presence of AMPS. In the pyloric region, the glandular cells of body region showed moderate reaction for AMPS. On contrary, Singh *et al.* (2012) reported moderate and strong AMPS reaction in supranuclear region of fundic and pyloric epithelium, respectively in group I. According to the authors, the AMPS activity increased with gestational age upto 40 cm CRL and in later stages of gestation, the activity decreased. AMPS activity could not be detected in fundic lamina propria, submucosa, tunica muscularis, serosa, blood vessels and nerve elements, whereas weak AMPS reaction was noticed in inter-glandular connective

tissue, blood vessels, tunica muscularis and tunica serosa of pyloric region (Table 1 & 2). Singh *et al.* (2012) observed moderate AMPS positive reaction in submucosa and strong activity in tunica muscularis of abomasum. Masot *et al.* (2007) could not observe acid mucopolysaccharide during development of red deer abomasum.

Bound lipids: Luminal border of glandular epithelium showed intense reaction in fundic and pyloric stomach (Fig. 8 & 10). Cytoplasm of parietal cell exhibited intense reaction in fundic region (Fig. 9). Smooth muscle cells of lamina muscularis and tunica muscularis, blood vessels revealed intense and moderate Sudan black B reaction in fundic and pyloric abomasum, respectively (Fig. 8 & 10). Presence of moderate to intense lipid activity was exhibited by nerve elements located in fundic as well as pyloric abomasum (Table 1 & 2). Singh *et al.* (2012) observed variable lipids deposition in the buffalo foetal abomasum.

Alkaline phosphatase (AKPS): In group III, blood vessels found in pyloric part of abomasum exhibited moderate alkaline phosphatase enzyme activity, elsewhere

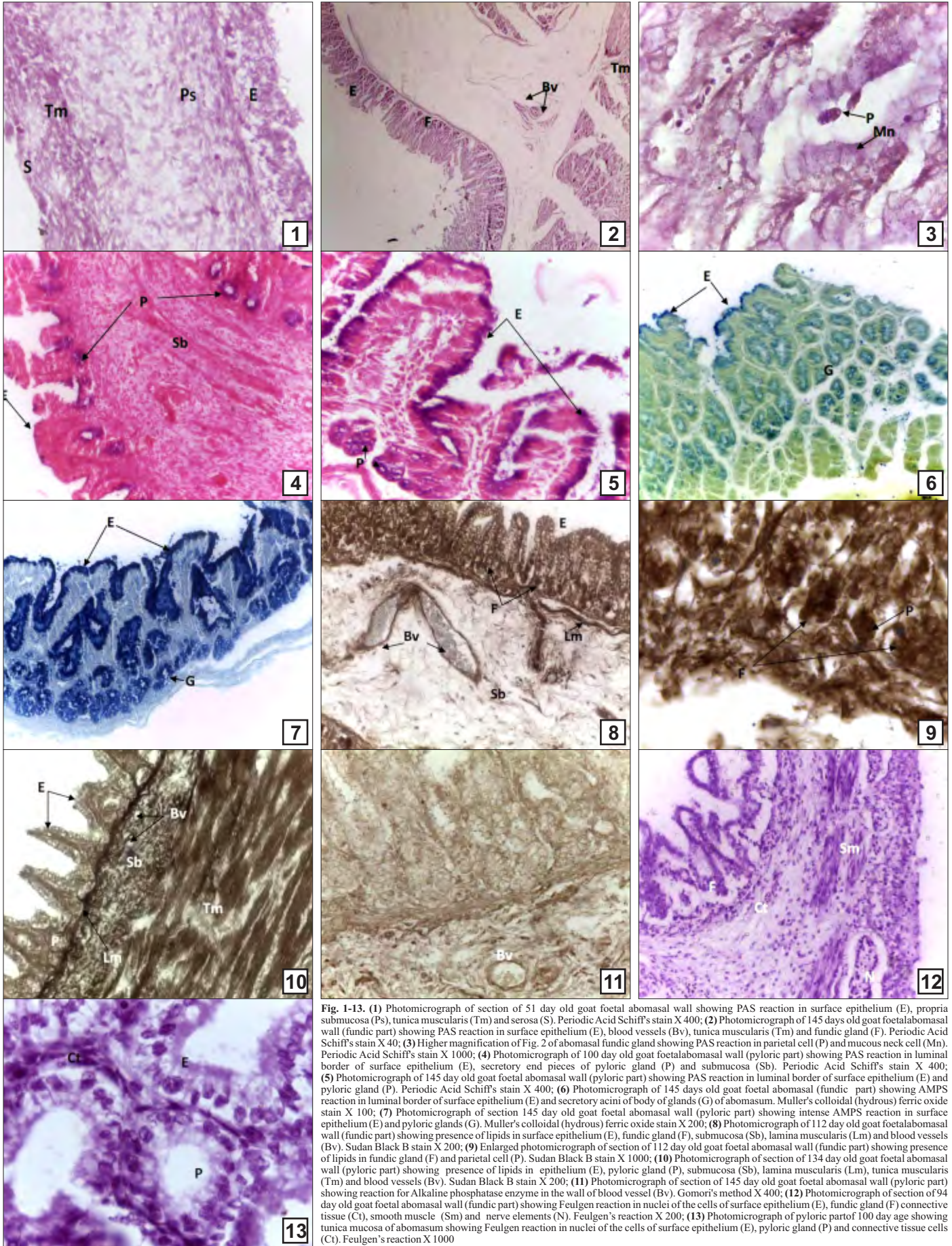


Fig. 1-13. (1) Photomicrograph of section of 51 day old goat foetal abomasal wall showing PAS reaction in surface epithelium (E), propria submucosa (Ps), tunica muscularis (Tm) and serosa (S). Periodic Acid Schiff's stain X 400; (2) Photomicrograph of 145 days old goat foetal abomasal wall (fundic part) showing PAS reaction in surface epithelium (E), blood vessels (Bv), tunica muscularis (Tm) and fundic gland (F). Periodic Acid Schiff's stain X 40; (3) Higher magnification of Fig. 2 of abomasal fundic gland showing PAS reaction in parietal cell (P) and mucous neck cell (Mn). Periodic Acid Schiff's stain X 1000; (4) Photomicrograph of 100 day old goat foetal abomasal wall (pyloric part) showing PAS reaction in luminal border of surface epithelium (E), secretory end pieces of pyloric gland (P) and submucosa (Sb). Periodic Acid Schiff's stain X 400; (5) Photomicrograph of 145 day old goat foetal abomasal wall (pyloric part) showing PAS reaction in luminal border of surface epithelium (E) and pyloric gland (P). Periodic Acid Schiff's stain X 400; (6) Photomicrograph of 145 days old goat foetal abomasal wall (fundic part) showing AMPPS reaction in luminal border of surface epithelium (E) and secretory acini of body of glands (G) of abomasum. Muller's colloidal (hydrous) ferric oxide stain X 200; (7) Photomicrograph of section 145 day old goat foetal abomasal wall (pyloric part) showing intense AMPPS reaction in surface epithelium (E) and pyloric glands (G). Muller's colloidal (hydrous) ferric oxide stain X 200; (8) Photomicrograph of 112 day old goat foetal abomasal wall (fundic part) showing presence of lipids in surface epithelium (E), fundic gland (F), submucosa (Sb), lamina muscularis (Lm) and blood vessels (Bv). Sudan Black B stain X 200; (9) Enlarged photomicrograph of section of 112 day old goat foetal abomasal wall (fundic part) showing presence of lipids in fundic gland (F) and parietal cell (P). Sudan Black B stain X 1000; (10) Photomicrograph of section of 134 day old goat foetal abomasal wall (pyloric part) showing presence of lipids in epithelium (E), pyloric gland (P), submucosa (Sb), lamina muscularis (Lm), tunica muscularis (Tm) and blood vessels (Bv). Sudan Black B stain X 200; (11) Photomicrograph of section of 145 day old goat foetal abomasal wall (pyloric part) showing reaction for Alkaline phosphatase enzyme in the wall of blood vessel (Bv). Gomori's method X 400; (12) Photomicrograph of section of 94 day old goat foetal abomasal wall (fundic part) showing Feulgen reaction in nuclei of the cells of surface epithelium (E), fundic gland (F) connective tissue (Ct), smooth muscle (Sm) and nerve elements (N). Feulgen's reaction X 200; (13) Photomicrograph of pyloric part of 100 day age showing tunica mucosa of abomasum showing Feulgen reaction in nuclei of the cells of surface epithelium (E), pyloric gland (P) and connective tissue cells (Ct). Feulgen's reaction X 1000

the activity was not found (Fig. 11; Table 1 & 2). Present observation was in partial agreement with Singh *et al.* (2012). These authors observed AKPS activity in the blood vessels of both in fundic as well as pyloric abomasum. The AKPS activity found in capillaries may be associated with process of active ion transport across the membrane (Dobson, 1959). Singh *et al.* (2012) also reported that the abomasal epithelium did not show any activity for AKPS in fundic and pyloric part however, weak activity was observed in basement membrane. Shrader and Zeman (1972) also failed to demonstrate AKPS activity in stomach of neonatal rat.

Acid phosphatase (ACPS): Acid phosphatase activity was detected neither in fundic nor in pyloric part of abomasum. Present observations disagreed with the findings of Singh *et al.* (2012) in buffalo foeti who observed strong ACPS activity in the fundic and pyloric epithelium and tunica muscularis in all groups. Dhote (1989) observed weak ACPS activity in buffalo calf upto 1 month in cardiac, fundic and pyloric part of abomasum. In the present study, ACPS activity could not be observed which might be due to species variation.

Deoxyribo-nucleic acid (DNA): Nuclei of the fundic as well as pyloric epithelial cells showed weak to moderate Feulgen reaction in all groups. Nuclei of chief and mucous neck cells and parietal cells exhibited moderate and intense Feulgen reaction, respectively. Intense and moderate Feulgen reaction was noticed in the nuclei of connective tissue cells of lamina propria and submucosa and endothelial cells of blood vessels of all groups in fundic and pyloric region, respectively (Fig. 12 & 13) (Table 1 & 2). Nuclear membrane and nucleoplasm of smooth muscle cells revealed intense and moderate Feulgen reaction, respectively in fundic part. The nuclei of supporting and ganglionic cells showed moderate and

intense Feulgen reaction in all groups in the fundic region, while it was moderate to intense in the pyloric region. DNA content observed in most of the nuclei of glandular cells and smooth muscle cells was suggestive of active stage of the cells.

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